NASA NAG 2-704 Prof. Alfred L. Goldberg

FINAL TECHNICAL REPORT

The primary goal of our research supported by NASA was to learn more about the mechanisms that activate protein breakdown in atrophying skeletal muscle. Accelerated proteolysis is a primary cause of muscle wasting induced by disuse, denervation, sepsis, and in several endocrine disorders. Prior studies had demonstrated three distinct proteolytic pathways in muscle: a lysosomal process, a Ca^{2+}-dependent process, and a cytosolic pathway that requires ATP. Our studies attempted to learn more about the regulation of these pathways and their relative importance in muscles in which proteolysis is accelerated due to disuse and high levels of glucocorticoids. Of particular interest was our discovery that the ATP-dependent process, involving ubiquitin and the proteasome rises upon denervation, adrenal hormones, fasting, and in other catabolic states, and can be the major cause of protein loss. We have explored the biochemical basis of this increased proteolysis and attempted to learn whether similar changes occur in other forms of muscle wasting.

Of appreciable importance has been our success in identifying small molecule inhibitors of the proteasome, i.e. the peptide aldehydes, MG115 (CBZ-LLnorV-al) or MG132 (CBZ-LLnorL-al), which can enter cells and retard protein breakdown in vivo. These agents reduce not only the rapid degradation of abnormal or short-lived proteins, but also inhibit the breakdown of long-lived proteins, which comprise the bulk of proteins in muscles and other mammalian cells. We have used these inhibitors to prove that this pathway is an important contributor to protein breakdown in isolated rat soleus muscles. Moreover, in muscles where the ubiquitin-proteasome pathway appeared to be activated, MG132 was more effective in suppressing protein breakdown. These approaches directly demonstrate that activation of the ubiquitin-proteasome process is mainly responsible for muscle wasting in denervation atrophy and in rats treated with high levels of glucocorticoids, thyroid hormones or metabolic acidosis, as was suggested by recent indirect studies. Consequently, these inhibitors and others that may affect this degradative pathway appear to have therapeutic promise to combat muscle atrophy of the kind seen in space personnel.

Related biochemical studies in muscle extracts demonstrated that the ubiquitin-proteasome pathway is the primary system for degrading endogenous soluble proteins, as well as the myofibrillar components (actin, myosin, troponin, tropomyosin), whose site of degradation had long been unclear. In extracts of rabbit psoas muscle, the complete degradation of soluble proteins to amino acids was stimulated up to 6-fold by ATP. Moreover, peptide aldehyde inhibitors of the proteasome or the removal of proteasomes by different centrifugation markedly inhibited the ATP-dependent process. Addition of purified myosin, actin, troponin or tropomyosin to these extracts showed that these proteins served as substrates for the ubiquiti-proteasome pathway. However, when myosin, actin and troponin were added as actomyosin complexes or as intact myofibrils to these extracts, they were not hydrolyzed at a significant rate, probably because in these multicomponent complexes, these proteins are protected from degradation.

Accordingly, actin (but not albumin or troponin) inhibited the degradation of ^{125}I-myosin, and actin was found to selectively inhibit ubiquitin-conjugation to ^{125}I-myosin. Also, the presence of tropomyosin inhibited the degradation of ^{125}I-troponin, but not ^{125}I-lysozyme or soluble muscle proteins. Thus, specific interactions between the myofibrillar proteins appear to protect them from ubiquitin-dependent degradation, and the rate-limiting step in their degradation is probably their dissociation from the myofibril.

During the final grant year, my laboratory made appreciable progress toward proposed research goals. A number of original articles and reviews have been completed and published (see below).

Publications pertinent to this grant:

Medina, R, Wing, SS and Goldberg, AL. Increase in levels of polyubiquitin and proteasome mRNA in skeletal muscle during starvation and denervation atrophy. Biochem. J (U.K.) 1995; 307: 631-637.

Wing, SS, Haas, AL and Goldberg, AL. Increase in ubiquitin-protein conjugates concomitant with the increase in proteolysis in rat skeletal muscle during starvation and denervation atrophy. Biochem. J (U.K.) 1995; 307: 639-645.

Baracos, VE., DeVivo, C, Hoyle, DHR. and Goldberg, AL. Activation of the ATP-ubiquitin-proteasome pathway in skeletal muscle of cachectic rats bearing a hepatoma. Am. J. Physiol 1995; E996-E1006.

Pepato, MT, Migliorini, RH, Goldberg, AL and Kettelhut, IC. Role of different proteolytic pathways in the degradation of muscle protein from streptozotocin-diabetic rats. Am. J. Physiol. 1996; E340-347.

Mitch, W and Goldberg, AL. Mechanisms of muscle wasting: the role of the ubiquitin-proteasome pathway. N. Engl. J. Med., 1996; 335: 1897-1905.

Alfred L. Goldberg, Ph.D.